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In situ assessment of lampricide toxicity to age-0 lake sturgeon

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ABSTRACT

The lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2′, 5-dichloro-4′-nitrosalicylanilide (niclosamide) are used to control sea lamprey (*Petromyzon marinus*), an invasive species in the Great Lakes. Age-0 lake sturgeon (*Acipenser fulvescens*), a species of conservation concern, share similar stream habitats with larval sea lampreys and these streams can be targeted for lampricide applications on a 3- to 5-year cycle. Previous laboratory research found that lake sturgeon smaller than 100 mm could be susceptible to lampricide treatments. We conducted stream-side toxicity (bioassay) and *in situ* studies in conjunction with 10 lampricide applications in nine Great Lakes tributaries to determine whether sea lamprey treatments could result in *in situ* age-0 lake sturgeon mortality, and developed a logistic model to help predict lake sturgeon survival during future treatments. In the bioassays the observed concentrations where no lake sturgeon mortality occurred (no observable effect concentration, NOEC) were at or greater than the observed sea lamprey minimum lethal concentration (MLC or LC99) in 7 of 10 tests. We found that the mean *in situ* survival of age-0 lake sturgeon during 10 lampricide applications was 80%, with a range of 45–100% survival within streams. Modeling indicated that in age-0 lake sturgeon survival was negatively correlated with absolute TFM concentration and stream alkalinity, and positively correlated with stream pH and temperature. Overall survival was higher than expected based on previous research, and we expect that these data will help managers with decisions on the trade-offs between sea lamprey control and the effect on stream-specific populations of age-0 lake sturgeon.

1. Introduction

Rehabilitation activities aimed at protecting critical habitats and life stages of lake sturgeon (*Acipenser fulvescens*) have yet to realize significant increases in population abundance, in part due to the level of reduction in abundance coupled with the life history of this species (Auer, 1999; Welsh et al., 2008). Presently, the lake sturgeon is listed as endangered or threatened in the vast majority of their historic range (Auer, 1999; COSEWIC, 2006). Sea lamprey (*Petromyzon marinus*) are an invasive species that contributed to the decline in many fish stocks throughout the Great Lakes (Siefkes et al., 2013) and spawn in streams that are also suitable for lake sturgeon spawning, with the natal habitat of soft sediments and sand being shared by both species (Kempinger, 1996; Peake, 1999).

Of over 5000 tributaries to the Great Lakes, 57 are known to support at least some life stages of lake sturgeon and an additional 40 are thought to have historical evidence of lake sturgeon (Table 1). Of these 97 rivers, 72 are known to be currently infested or have had at least had one sea lamprey infestation since the beginning of sea lamprey surveys in the Great Lakes, and 46 of these receive lampricide applications on a regular (3–4) year cycle (Table 1). Sea lampreys are controlled in Great Lakes tributaries and estuaries by the application of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and 2′,5-dichloro-4′-nitrosalicylanilide (niclosamide) (Siefkes et al., 2013). Year- and stream-specific pH and alkalinity measures affect the toxicity of TFM to aquatic organisms (Bills et al., 2003). Alkalinity and pH data of a tributary are required to calculate the minimum amount of TFM required to kill 99.9% (LC99, or minimum lethal concentration, MLC) of sea lamprey larvae in the tributary. TFM application rates are typically 1.2–1.5 times the MLC to ensure that treatment efficacy is not affected by attenuation or dilution of lampricide as it moves downstream, with the goal of maintaining at least 9 h of exposure at or above the MLC throughout the length of infested stream. Niclosamide can be used in conjunction with TFM, typically at a rate of up to 1% by weight of active ingredient of the TFM applied, to reduce the target MLC for sea lampreys (Bills and Marking, 1976). Consequently, this reduces the amount and subsequent cost of TFM required to control larval sea lampreys, and is most often used in tributaries with pH > 7.0 or when the addition of niclosamide results in a substantial savings in cost (Gutreuter and Boogaard, 2007).

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Table 1
Summary of current and historical lake sturgeon streams in the Great Lakes with current or historical sea lamprey streams. Sea lamprey streams include those that have had at least one positive sea lamprey survey. Regular sea lamprey treatments are those that are conducted on a three to four year cycle, while irregular sea lamprey treatments are those ranging from a single treatment to those on a 5 to 10 year cycle.

Lake	Total streams	Current lake sturgeon streams	Historical lake sturgeon streams	Total lake sturgeon streams with sea lampreys	Lake sturgeon streams with regular sea lamprey treatments	Lake sturgeon streams with irregular sea lamprey treatments
Lake Superior	1566	16	6	21	10	11
Lake Huron	1761	16	17	25	18	7
Lake Michigan	511	17	10	18	15	3
Lake Erie	842	3	5	3	1	2
Lake Ontario	659	5	2	5	2	3
Total	5339	57	40	72	46	26

The effect of lampricides on non-target fishes has been a concern since the development of the lampricide application program in the 1960s (Applegate and King, 1962; Bills and Marking, 1976; Marking and Olson, 1975), and, more recently, specifically for lake sturgeon (Boogaard et al., 2003; Johnson et al., 1999). Steam pH was the primary factor in determining TFM toxicity to juvenile lake sturgeon 100 to 125 mm total length, but there was no significant mortality for this size group when lampricides were applied at a rate of 1.3× MLC or less (Johnson et al., 1999). Further, lake sturgeon sac fry and fingerlings > 125 mm were the most resistant to TFM, but that swim-up fry and fingerlings < 100 mm were more susceptible than most teleosts when exposed to TFM at minimum lethal concentrations for sea lampreys (Boogaard et al., 2003).

Due to concerns about mortality of age-0 lake sturgeon < 100 mm in total length, a protocol for lampricide applications in U.S. tributaries with known lake sturgeon populations was developed to 1) restrict the amount of TFM applied to 1.0× MLC and TFM/niclosamide to 1.2× MLC, and 2) ensure that treatments of these streams occurred after August 1, when the majority of lake sturgeon are expected to be >100 mm in length (Adair and Sullivan, 2009). However, application of lampricides late in the year and at reduced concentrations has raised concerns among sea lamprey control managers for several reasons. First, prior to adopting the revised treatment protocol, field personnel had observed only 10 dead lake sturgeon in over 16,000 post-treatment collections following 1800 lampricide stream treatments that occurred from 1959 to 2000 (Johnson et al., 1999). Between 2001 and 2012, an additional 982 lampricide treatments have been conducted and only 3 dead lake sturgeon were observed during this time (unpublished USFWS and DFO treatment reports). During the supplemental lampricide applications that are conducted during lampricide treatments (Adair and Sullivan, 2009), survey crews cover the entire length of sea lamprey infested portion of the river, looking for sea lamprey escapement areas. During these surveys, crews look for both sea lamprey and any non-target species mortalities, paying special attention to any lake sturgeon mortality (Adair and Sullivan, 2009). Because larval sea lamprey and lake sturgeon lack swim bladders, both sink to the bottom when dead; thus crews used to surveying stream bottom for sea lamprey mortality are skilled at looking for affected lake sturgeon. Nevertheless, extensive effort by the U.S. Fish and Wildlife Service, Little River Band of Ottawa Indians, and the Michigan Department of Natural Resources found 31 dead age-0 lake sturgeon during the 2014 lampricide treatment of the Muskegon River, (S. Nowicki, USFWS, 2015, personal communication); more than all other stream treatments combined. This indicated that lampricide-induced mortality of age-0 lake sturgeon could be greater than previously observed and that discovery of dead age-0 lake sturgeon may require a concerted effort. Second, since the revised treatment protocol was adopted on lake sturgeon producing streams, wounding rates among native fishes and population estimates of larval and spawning sea lampreys in the upper Great Lakes have increased (Slade, 2012; Sullivan et al., 2013). Lastly, survival of sea lamprey larvae in lake sturgeon producing streams was greater following treatments conducted in late September and beyond when the revised protocol was followed compared to earlier in the season (Scholefield et al., 2008). As a result, treatments were required more frequently on some large rivers, in some cases every one or two years, compared to their normal treatment cycle of once every three to four years, due to the number of these residual sea lampreys (Boogaard et al., 2011). When all these points are combined, the concern was that management actions taken to protect lake sturgeon may have resulted in increased sea lamprey production, increased treatment frequency of large rivers, and subsequently increased the frequency of exposure of age-0 lake sturgeon to TFM in these rivers.

The adherence to the restricted lampricide application protocols may not result in the expected benefits to lake sturgeon survival because observations of lake sturgeon mortality in laboratory tests do not correspond to the in-stream observations during and immediately following lampricide application. Instead, reduced lampricide application rate may increase the production of sea lampreys to the Great Lakes thereby increasing the likelihood of sea lamprey induced mortality on older lake sturgeon (Patrick et al., 2009), and more frequently expose cohorts of age-0 lake sturgeon to lampricide applications. During 2010 and 2011, we conducted a study to better understand the disparity between laboratory and field observations, and provide *in situ* observations of lake sturgeon exposed to lampricide application. Our specific research objectives were to 1) compare the calculated toxicity of TFM or TFM/niclosamide based on pH and alkalinity measures with observed mortality of age-0 lake sturgeon and sea lampreys in controlled, pre-treatment bioassays; 2) evaluate *in situ* mortality of age-0 lake sturgeon held in cages during TFM and/or TFM/niclosamide treatments; and 3) develop a predictive model of lampricide-induced, age-0 lake sturgeon mortality based on stream-specific lampricide applications to kill sea lamprey larvae. Based on the most recent bioassay studies (Boogaard et al., 2003; Johnson et al., 1999), we expected moderate to high lake sturgeon mortality *in situ* and in the bioassays when TFM and TFM/niclosamide concentrations exceeded the MLC for sea lampreys.

2. Methods

2.1. Study sites

Site selection was based on the criteria that the streams: 1) were scheduled to be treated with TFM or TFM/niclosamide during 2010 or 2011; 2) represented a range of discharge, pH and alkalinity values typically encountered during lampricide applications (Table 2); and 3) where possible, were used by lake sturgeon for spawning. Streams that met this criteria were: the Kaministiquia River and its independently treated tributary, the Whitefish River, and the Batchawana and Two-Hearted rivers (Lake Superior); the Mississagi, Rifle and Pigeon rivers (Lake Huron); and the Millecoquins, and Sturgeon rivers (Lake Michigan; Fig. 1). The Rifle River was treated in two separate parts; the upper section was treated exclusively with TFM and the lower section with TFM/niclosamide. The two sections were treated as independent observations and were assessed separately, resulting in 10 treatments to evaluate *in situ* lampricide-induced lake sturgeon mortality.

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Table 2
Study rivers treated with TFM or TFM/niclosamide in 2010 and 2011, including treatment dates, type, discharge, and length of river treated with lampricide. Minimum lethal concentration (MLC) chart (mg/L) was calculated using the pH and alkalinity values at the start of the lampricide treatment (Bills et al., 2003); Application Target (mg/L) was the amount of TFM or TFM/niclosamide applied to river.

Tributary	Lake	Treatment date	Treatment type	pH	Alkalinity (mg $CaCO_3$/L)	Discharge (m3/s)	MLC Chart (mg/L)	Application target (mg/L)	Distance treated (km)
Two-Hearted	Superior	06/08/2010	TFM	7.72	63	5	1.3	1.7	95
Millecoquins	Michigan	22/08/2010	TFM	8.56	102	3.1	5.0	5.0	15
Sturgeon	Michigan	03/09/2010	TFM	7.79	82	6.5	1.0	2.0	40
Whitefish	Superior	08/09/2010	TFM	8.08	86	0.6	2.0	3.1	21
Kaministiquia	Superior	11/09/2010	TFM/1% niclosamide	7.79	47	29.3	1.0	1.6	54
Rifle (upper)	Huron	09/08/2011	TFM	8.12	241	4.8	4.4	9.0	129
Batchawana	Superior	17/08/2011	TFM	7.79	25	4.2	1.0	1.6	12
Pigeon	Huron	06/09/2011	TFM	8.27	193	3.4	5.0	7.1	54
Mississagi	Huron	21/07/2011	TFM/1% niclosamide	7.39	25	65.8	0.6	0.8	49
Rifle (lower)	Huron	08/08/2011	TFM/0.5% niclosamide	8.59	192	4.8	5.0	5.3	65

2.2. Juvenile sturgeon culture

Rainy River strain lake sturgeon were obtained as eggs or sac fry from Sustainable Sturgeon Culture (Emo, Ontario). This strain was used in previous toxicity tests (*e.g.*, Boogaard et al., 2003) because Great Lakes strain lake sturgeon are not currently commercially available. Lake sturgeon are a controlled species under Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) legislation, making transport across the international border difficult. Consequently, fish were reared in separate facilities in Canada and the United States. Lake sturgeon eggs were transported to the Genoa National Fish Hatchery, in Genoa Wisconsin, while Fisheries and Oceans Canada (DFO), in Sault Ste. Marie, Ontario, received sac fry hatched by Sustainable Sturgeon Culture. Fish in both facilities were reared following the Genoa National Fish Hatchery Lake Sturgeon Culture Standard Operating Procedures manual (Aloisi et al., 2006). After hatch and swim up, lake sturgeon from Genoa were transported to the U.S. Geological Survey's Upper Midwest Environmental Sciences Center and reared at similar water temperatures as the DFO facility to maintain a similar size range between the two rearing locations. Lake sturgeon were fed brine shrimp and blood worm (as appropriate for size, following Aloisi et al., 2006) *ad libitum* in the hatchery. Once randomly selected for either the toxicity or *in situ* experiments, fish were no longer fed once placed in either the aquaria (toxicity experiments) or cages (*in situ* experiments).

2.3. Toxicity tests

Toxicity tests were conducted streamside in mobile bioassay trailers. Exposures were conducted in a ten-cell continuous flow-delivery system following the protocol outlined in Technical Operating Procedures for Application of Lampricides (Adair and Sullivan, 2009). The concentrations of the aquaria were set in an 85% dilution pattern (80% dilution pattern was used by USFWS), such that the concentration in aquarium 2 was 85% that of aquarium 1, and the concentration in aquarium 9 was 27% that of aquarium 1. Aquarium 10 (the control) maintained a 0% chemical concentration. Stock solutions of TFM and TFM/niclosamide were prepared according to methods in Adair and Sullivan (2009), and tests were comprised of either TFM or TFM/niclosamide based on the planned treatment for each river.

Age-0 lake sturgeon and larval sea lamprey were introduced into the aquaria approximately 18 h prior to the toxicity test (Adair and Sullivan, 2009). Lake sturgeon were randomly drawn from the pool of fish available at the time of treatment, and distributed in groups of five or ten into each aquarium. Ten larval sea lampreys were added to each aquarium with the exception of the Batchawana River treatment, where larval sea lampreys were unavailable for the bioassay. Lake sturgeon were fed *ad libitum* in the hatchery and larval sea lampreys fed in the stream environment until both species were placed in the aquaria, then not fed again. Test animals were exposed to the lampricides for 12 h. Within each aquarium, TFM concentration, water temperature, and pH were measured hourly, and niclosamide concentration every two hours. Dissolved oxygen was measured at hours two, five and eight, and total alkalinity measured at hours three, six and nine. Both species were examined for mortality hourly during the 12 h exposure and at 12 h post-exposure to check for delayed mortality. Length and weight information on dead lake sturgeon was collected differently among years. During 2011, and for the Kaminisitiquia and Whitefish rivers during 2010, all dead lake sturgeon were measured ($\pm$1 mm) and weighed ($\pm$0.1 g) after the hourly mortality assessment and the remainder at the conclusion of the 12 h post-exposure period. During 2010, a subsample of 20 fish, a mix of live and dead subjects, were measured in the bioassays for the Two-Hearted, Millecoquins, and Sturgeon rivers. All fish were euthanized following bioassay.

We assigned the observed sea lamprey MLC (MLC_{obs}) as the concentration in the tank with 100% sea lamprey mortality at the end of the 12-h exposure. We also assigned the lake sturgeon No Observable Effect Concentration (NOEC) from the tank that contained 100% lake sturgeon survival following the 12 h lampricide exposure (Table 3). Lastly, we recorded lake sturgeon survival in the tank where we observed full sea lamprey mortality (MLC_{obs}).

2.4. In situ lampricide application

Application of TFM or TFM/niclosamide to the tributaries was conducted within seven days of the bioassay, following standard operating procedures (Adair and Sullivan, 2009). The lampricide application concentration for the individual tributaries was determined by the water chemistry at the time of treatment, following the pH/alkalinity prediction charts (MLC Chart; Bills et al., 2003). Lampricide was initially applied at a rate of up to 2$\times$ MLC (MLC Target and MLC Application Rate; Table 2), the regular lampricide treatment protocol, to compensate for attenuation and dilution as the chemical block moved downstream. Water chemistry (pH and total alkalinity), TFM or TFM/niclosamide concentration measurements were taken at standardized water sampling locations along the length of each tributary during the treatment. Lampricide application on the Pigeon River differed between the bioassay and stream treatment. The bioassay was conducted with TFM/1% niclosamide which was the original plan for the treatment of the river. However, a rain event changed the river's chemistry and discharge, thus only TFM was applied during the treatment.

One-hundred and fifty age-0 lake sturgeon were randomly chosen from the culture facility; 100 were assigned to the lampricide treatment group, and 50 were assigned to the control group. This resulted in five lake sturgeon in each of the 20 cages (41 cm $\times$ 23 cm diameter minnow traps with the funnels sealed using window screening) placed in the treated section, and 10 cages positioned in the control section of each tributary.

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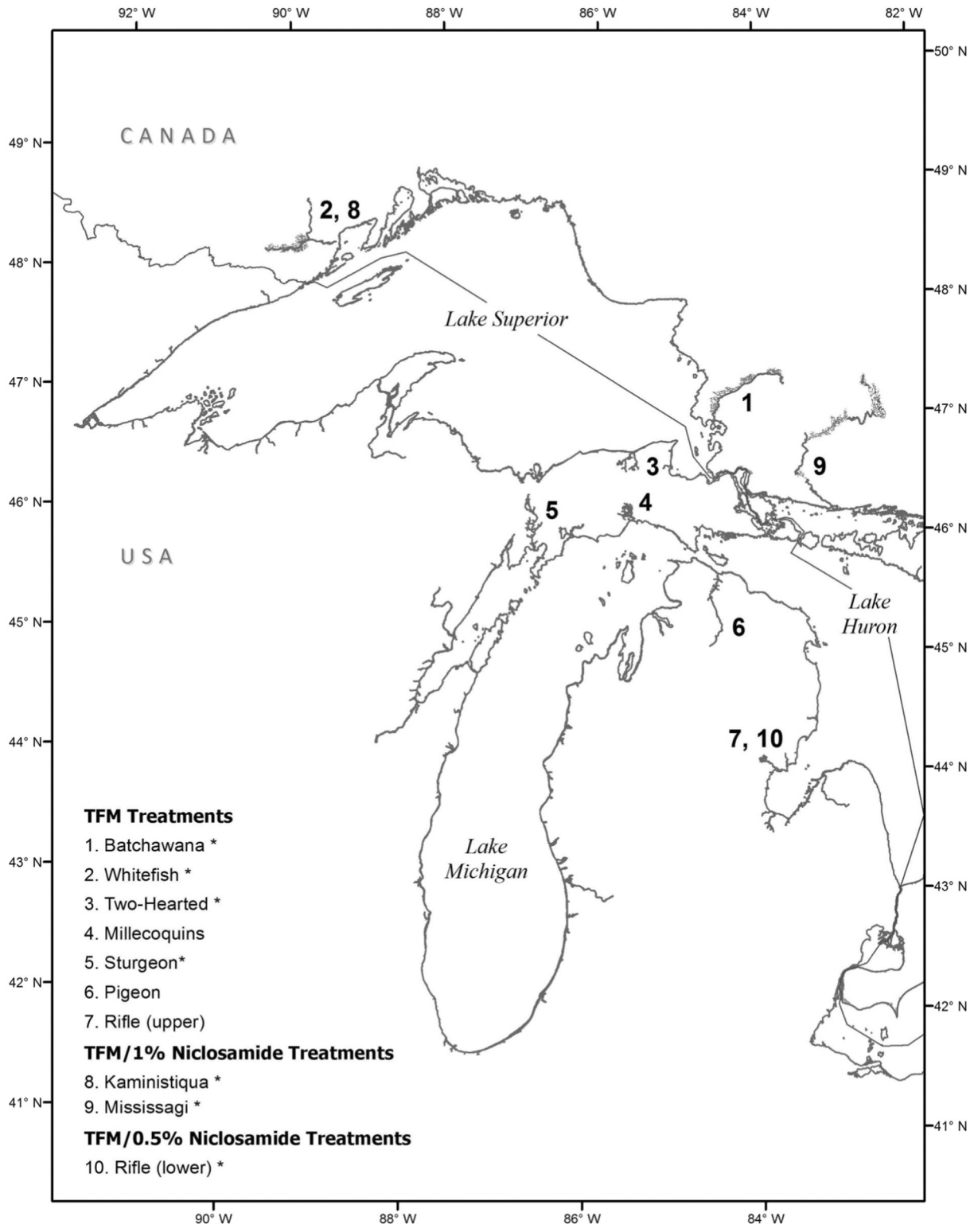

Fig. 1. Map of Great Lakes tributaries in this study indicating treatment type and streams containing extant or had historical lake sturgeon are indicated with an asterisk.

Cages were randomly allocated to sites throughout the treatment area. Control cages were placed in association with, but upstream of, the application point, and all cages were situated in a manner that ensured appropriate water flow and depth to minimize any additional stress on the fish. Sturgeon were acclimated to each stream for 24 h prior to the treatment, treatment cages were exposed to the lampricide(s) for the duration of treatment (typically 12 to 14 h) and then all cages were removed 24 h post-treatment. At the time of retrieval, fish were measured (±1 mm), weighed (±1 g), and recorded as alive or dead. No lengths or weights were recorded from the *in situ* cages in the Two-Hearted, Millecoquins, and Sturgeon rivers during 2010, so fish measured in the bioassay sub-samples for these three rivers were used as surrogates as the fish were randomly drawn from the same source of available subjects and the stream treatment occurred within 7 days of the bioassay.

Logistic issues, lake sturgeon availability, vandalism, and other limitations resulted in reductions in the number of treatment and control

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Table 3
Summary of stream-side bioassay results for 2010 and 2011 by treatment type and date. Tributaries with aor b under Test indicate the use of a 20 tank, dual bioassay trailer which allowed two bioassays with overlapping but different concentrations. The pH and alkalinity values at the start of the bioassay were used to determine the MLC (mg/L) for larval sea lampreys (Bills et al., 2003). The lake sturgeon LC50 and the sea lamprey MLC were calculated at the conclusion of the bioassay using the R statistical package *LW1949*. No larval sea lampreys were available for the bioassay for the Batchawana River.

Tributary	Test	Lampricide	pH	Alkalinity (mg $CaCO_3$/L)	Sea Lamprey			Lake Sturgeon	
					MLC (mg/L)	MLC Observed (mg/L)	MLC Calculated (mg/L)	NOEC (mg/L)	LC50 Calculated (mg/L)
Kaministiqua	a	TFM/Niclosamide	7.96	48	0.9	1.22	1.54	1.22	na
Kaministiqua	b	TFM/Niclosamide	7.98	47	0.9	1.12	1.47	0.97	na
Mississagi	a	TFM	7.52	25	0.8	0.60	0.58	0.60	0.97
Mississagi	b	TFM	7.52	25	0.8	0.58	na	0.78	0.92
Batchawana	a	TFM	7.59	25	0.9	na	na	1.17	1.41
Batchawana	b	TFM	7.66	27	1.0	na	na	1.07	na
Two-Hearted	a	TFM	7.47	53	0.9	1.50	1.56	2.30	na
Millecoquins	a	TFM	8.31	107	3.6	4.60	4.35	3.70	5.98
Pigeon	a	TFM/Niclosamide	8.17	203	2.6	3.01	2.91	3.68	4.09
Rifle (upper)	a	TFM	8.14	198	4.0	4.16	4.57	3.40	4.71
Rifle (lower)	a	TFM/Niclosamide	8.27	197	2.9	2.85	2.80	2.85	3.24
Sturgeon	a	TFM	7.84	98	1.9	2.90	4.02	2.90	na

cages in some instances. For example, only 7 control cages were deployed in the Two-Hearted, Millecoquins, and Sturgeon rivers. Control cages in the Whitefish River were removed 12 h early (36 h *vs*. 48 h) due to a miscommunication with the field crew. Five of the cages were lost due to vandalism (two each in the Two-Hearted and upper Rifle rivers, one in the Kaministiquia River) and one cage was tampered with but not removed from the treatment zone in the Kaministiquia River. All control fish in the Mississagi River were lost; four of the control cages were removed by vandals, and the remaining control cages were inadvertently placed in the portion of the river that was dewatered when the water levels were reduced 2 m overnight during the treatment.

In total, of the 1005 age-0 lake sturgeon placed in cages in the rivers, 107 were not recovered post treatment. Fish loss was not equal among treatments. For the majority of the treatments (60%), fish escapement was 0%, and in one river fish loss was <5%. The majority of the escapement occurred during the Mississagi River (30%) and upper Rifle River (69%), which accounted for 86% of the escaped fish loss (N = 92). In the Mississagi River mean fish size was 40 mm (27–47 mm), thus the fish cages were screened with an inner screening to retain fish; however, there was still escapement. The Mississagi River treatment occurred in mid- July, while the other rivers were treated in August or September (Table 2). The lake sturgeon, which were growing at a natural rate, were of smaller size compared to those fish used one month later. However, they were similar in size to native lake sturgeon collected in the Mississagi River in late July 2008 (mean 50 mm) (L. O'Connor, unpublished data). Unrecovered fish were not assumed to have either survived or died during the treatments, and were removed from any survival analyses.

3. Data analyses

3.1. Toxicity tests

For those toxicity tests where the full range of survival (at least one tank with 100% survival and one tank with 100% mortality) was observed for either lake sturgeon or sea lamprey, we used the automated Litchfield-Wilcoxon method package LW1949 in R to calculate the concentration of TFM or TFM/niclosamide that would produce 50% mortality (LC50) in lake sturgeon and the LC99 (MLC) for sea lamprey. We then used linear regression to compare among the sea lamprey MLC forecast from pH and alkalinity data (Bills et al., 2003), and the R-calculated sea lamprey MLC and lake sturgeon LC50. A slope significantly different from unity in the regression indicates a significant difference in the toxicities being compared among all toxicity tests in the study.

3.2. In situ tests

We conducted several separate, single-variable tests to evaluate effects on lake sturgeon mortality. Independent sample *t*-tests were used to examine: a) river-specific differences in mean length among lake sturgeon placed in control and treatment cages, and; b) to determine if length was a factor in survival among lake sturgeon placed in the treatment section of the stream. Second, Bonferroni-corrected log-linear analyses (Zar, 1999) were used to evaluate the effects of length on *in situ* mortality of age-0 lake sturgeon held in cages during TFM and TFM/niclosamide treatments. Third, separate log-linear analyses contrasted lake sturgeon survival among the control and treatment areas in each of the tributaries. The Mississagi River was excluded from this assessment due to the catastrophic loss of the control fish (see results). Lastly, we used ANOVA to determine the extent of cage mortality in the control cages as a function of the duration, in hours, that control cages were placed in the river. The Mississagi River control cages were also excluded from this analysis.

3.3. Predictive model

A generalized linear model using a logit link function was developed in R to determine factors affecting lake sturgeon survival. As TFM concentration was not measured at or within each sturgeon cage, cage results were grouped within streams in association with the closest TFM sampling site or grouped as control cages above the lampricide application point. Control cages from the Mississagi River treatment were not included in the analysis due to the known, non-treatment related mortality of this group of lake sturgeon. The mean survival among cages within each group was used as the response variable in analysis. Other site-and cage-specific parameters, including pH, alkalinity, duration of exposure to lampricide, TFM and niclosamide concentrations, duration that the cage was left in the river, and mean length of lake sturgeon in each cage were included as potential covariates.

Significant ($\alpha = 0.05$) main effects (Table 4) and all two-factor interaction terms of significant main effects were evaluated for inclusion in the model. Those that were statistically significant and biologically meaningful were included in further modeling. Collinearity among some terms was expected; for example, since the concentration of lampricide is predicated upon stream-specific measures of pH and alkalinity, we expected that the pH and alkalinity variables would be highly correlated with the target MLC for treatment. As well, niclosamide is applied as a percentage of the TFM target and niclosamide concentration will be correlated with both the MLC and the measures used to establish the target. Terms with variance inflation factors exceeding five were

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Table 4
Variables investigated in the predictive model of age-0 lake sturgeon survival during lampricide applications.

Variable	Description
Tributary	Tributary used
Year	Year of treatment
Cage type	Control/Treatment
Set Duration	The total amount of time the cage was in the river, in hours
Alive	Number of lake sturgeon that were alive per cage
Dead	Number of lake sturgeon that were dead per cage
pH	Average pH at site during TFM presence
Alkalinity	Average alkalinity at site during TFM presence, in mg $CaCO_3$/L
Temperature	Mean temperature during lampricide exposure
Niclosamide concentration	Concentration of niclosamide, in µg/L
MLC Treatment Target	The minimum lethal concentration of TFM for a 9-h exposure, calculated from stream-specific pH and alkalinity values immediately prior to treatment, in mg/L
Concentration Measured	Actual TFM concentration at site, in mg/L
TFM Duration	Exposure time at or exceeding MLC for each cage, in hours
Mean.Length	Mean length of lake sturgeon that survived treatment, in millimeters
Mean.Weight	Mean weight of lake sturgeon that survived treatment, in grams
TFM.difference	The ratio of Conc.Measured/MLC.Treatment.Target
Niclosamide Ratio	The ratio of niclosamide to TFM in the treatment; targeted to be either 1% or 0.5% by volume of TFM

removed from the model. The final model was used to forecast lake sturgeon survival for pH 8.1 (the mean of the *in situ* streams) with increasing alkalinity using the MLC values drawn from the pH/alkalinity tables that are used to set the current treatment targets for TFM and TFM/niclosamide. We used an application concentration ratio of 1.4 × MLC in the profile, similar to the mean initial application rate in current treatments.

4. Results

4.1. Toxicity tests

For those LC values that could be calculated, there was reasonable concordance between the R-calculated sea lamprey MLCs and the pH MLCs predicted from the pH/Alkalinity predication charts (Table 3; Fig. 2). The slope of the regression line comparing R-calculated MLC values with MLC based on pH and alkalinity was not significantly different than unity (1.29; 95% CI 0.74–1.85; $r^2 = 0.77$). Alternatively, the lake sturgeon LC50 *versus* the pH/alkalinity predicted MLC had a slope significantly >1 (1.46; 95% CI 1.08–1.85; $r^2 = 0.76$), indicating that the likelihood of lake sturgeon survival is >50% when exposed to the larval sea lamprey MLC. This is corroborated through direct observations of lake sturgeon NOEC (Table 3), where seven of ten bioassays had lake sturgeon NOEC values equal to or greater than the MLC of the tank where all sea lamprey died, and lake sturgeon mortality in the three remaining MLC tanks was limited to one fish each. No latent mortality (after 12 h post-exposure) was observed in lake sturgeon in any of the bioassay trials.

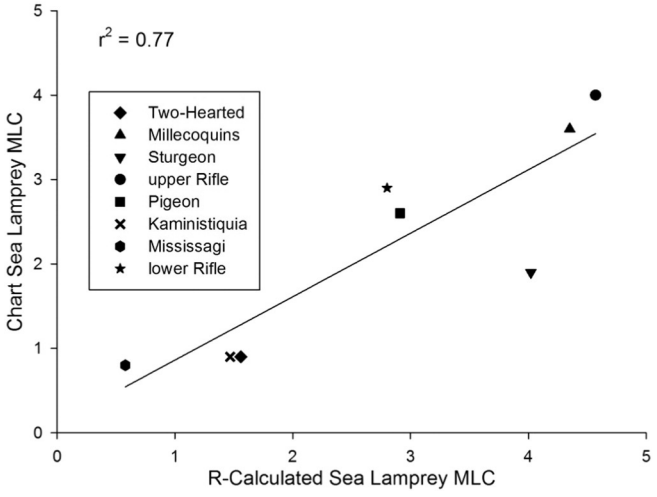

Fig. 2. The calculated minimum lethal concentration (MLC) of TFM or TFM/1% niclosamide from the bioassay study (R-calculated on figure) plotted against the same estimate from the published Bills et al. (2003) tables (Chart on figure).

4.2. In situ tests

A total of ten *in situ* lampricide applications to tributaries were completed during 2010 and 2011. Age-0 lake sturgeon *in situ* survival ranged from 45% to 100% in the treated sections of rivers (Table 5); mean age-0 survival in treatment areas was 78.8%. In five of the seven rivers, lake sturgeon were not significantly different in size between the treatment and control cages (*t*-tests, $p > 0.05$, Fig. 3).

Age-0 lake sturgeon in the *in situ* treatments ranged in size from 25 to 120 mm (Table 5), with 91% of the fish <101 mm in length. Lake sturgeon that survived treatments ranged from 35 to 120 mm total length, including 83% survival in the Mississagi River treatment where all fish were <50 mm in size (Table 5). We found that the mean length of fish that died was significantly smaller than the mean length of the surviving fish in the treatment section in four of seven tributaries, (*t*-tests, $p < 0.05$; Table 5, Fig. 4). Survival was significantly lower in five *in situ* treatment areas *versus* control areas (Table 5). Nevertheless, survival was 98.7% among the control cages (excluding the Mississagi River), and analysis of the duration, in hours, that the cage was in the stream indicated no substantial mortality due to cage effect (ANOVA, $F_{1, 79} = 0.109$, $p = 0.74$). Because control and treatment cages were handled in the same manner, including duration of set for all rivers except the Whitefish where control cages were lifted 12 h early, all lake sturgeon mortality in the treatment section was subsequently classified as the effect of lampricide application for use in the predictive model.

4.3. Predictive model

As expected, we found high collinearity among some variables. Removal of the MLC term as a main effect resulted in all other main effects having variance inflation factors less than five. The MLC term remains in the model as part of a calculated variable. No interaction terms were statistically significant and biologically meaningful. The final model includes main effects for pH, alkalinity, temperature, TFM concentration, minimum lethal concentration, and the ratio of measured to chart MLC (*i.e.* the multiplicative factor identifying the amount of TFM exceeding MLC) (Table 6), and takes the form:

$$\text{Logit}(\text{Lake Sturgeon Mortality}) = -17.380 + 2.838 * \text{pH} - 0.018 * \text{Alkalinity} + 0.074 * \text{Temperature} - 0.195 * \text{TFM Concentration} - 2.091 * (\text{TFM Concentration}/\text{Chart MLC})$$

When converted to a probability, the model shows that as alkalinity increases and temperature decreases, predicted survival for lake sturgeon decreases in both TFM and TFM/1% niclosamide treatments, (Fig. 5, Table 7).

5. Discussion

In general, we found that age-0 lake sturgeon mortality is positively correlated with the absolute amount of TFM that is applied to the stream. Environmental factors that determine TFM toxicity, such as alkalinity and pH, and subjective decisions by sea lamprey control

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Table 5
Summary of *in situ* treatment data for 2010 and 2011 by treatment type for the number of cages recovered in each river including the mean proportion live, mean lengths (and range in parentheses below) for both live and dead fish in the treatment and control cages. In 2010 only 20 age-0 lake sturgeon were measured during the bioassay for length and weight for the Two-Hearted, Millecoquins, and Sturgeon Rivers; this mean length has been applied to the *in situ* cage study. A single * indicates cages where the mortalities were significantly smaller in length (*t*-tests, $p < 0.05$) than those recovered live. A double ** indicates significant survival differences (log-linear analysis) after Bonferroni corrections between treatment and control fish.

Treatment	Tributary	Treatment						Control						Maximum likelihood χ^2
		N cages	Mean survival (%)	Live		Dead		N cages	Mean survival (%)	Live		Dead		
				N	Length (mm)	N	Length (mm)			N	Length (mm)	N	Length (mm)	
TFM	Two-Hearted	20	100	100	85 (72–106)	0		7	100	35		0		$\chi^2 = 0.2$, $p = 0.62$
	Millecoquins	21	92	97	95 (63–115)	8		7	100	35		0		$\chi^2 = 2.6$, $p = 0.11$
	Sturgeon	21	80	88	93 (78–120)	22		7	94	33		2		$\chi^2 = 4.0$, $p = 0.05$
	Whitefish	20	80	78	95 (75–114)	20	81* (63–113)	10	100	50	94 (71–115)	0		$\chi^2 = 15.7$, $p < 0.001$**
	Upper Rifle	18	67[a]	24	74 (66–88)	14	71 (61–80)	10	98	47	73 (62–85)	1	67	$\chi^2 = 15.7$, $p < 0.001$**
	Batchawana	20	88	88	73 (62–87)	12	70 (64–78)	10	98	49	74 (57–84)	1	71	$\chi^2 = 4.3$, $p = 0.04$
	Pigeon	20	45	40	86 (69–99)	49	82 (64–96)	10	100	50	84 (62–103)	0		$\chi^2 = 30.3$, $p < 0.001$**
TFM/niclosamide	Kaministiquia	25	88	107	98 (62–128)	14	83* (64–108)	10	100	49	90 (61–115)	0		$\chi^2 = 7.2$, $p = 0.007$**
	Mississagi	20	83	57	42 (35–47)	12	36* (29–42)	6	0	0		25	34[b] (25–42)	
	Lower Rifle	20	65	62	72 (62–83)	34	68* (53–84)	10	98	47	71 (62–82)	1	68	$\chi^2 = 16.7$, $p < 0.001$**

[a] Proportion live of those lake sturgeon recovered: 7 cages were empty and a total of 62 sturgeon were missing at the end of the treatment.
[b] No live controls were recovered from the Mississagi River treatment: 4 cages were vandalized and control area was subjected to dewatering due to hydroelectric demands, killing all remaining fish: fish were dead approximately 24 h prior to length and weight measurements.

treatment personnel regarding the amount of TFM applied in excess of the pH- and alkalinity-based amount (to address attenuation of TFM concentration) are all correlated with lake sturgeon mortality. These effects are reduced as stream temperature increases. All of these factors are contained within the logistic model of survival based upon the *in situ* data.

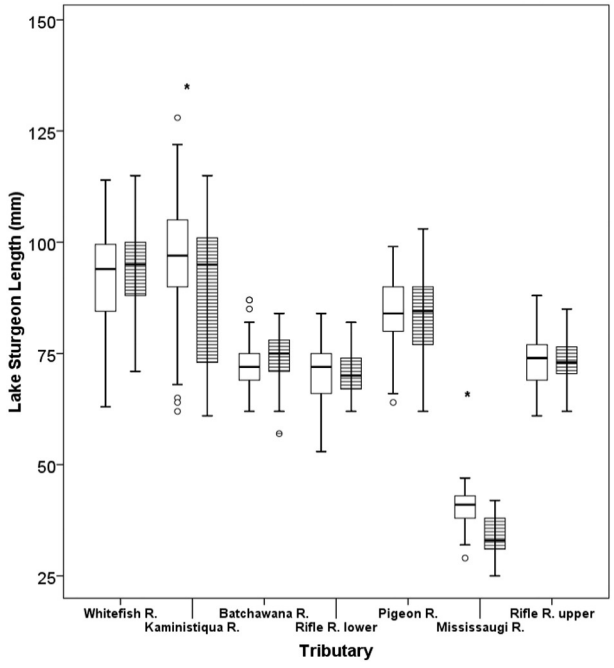

Fig. 3. Box plot comparing lake sturgeon length (mm) among treatment and control sections for seven of the treated tributaries; The Two-Hearted, Millecoquins, and Sturgeon rivers were not included as individual lake sturgeon lengths were not recorded in these rivers. Lake sturgeon were significantly larger in the Kaministiquia and Mississagi treatment sections; noted with *. Treatments are represented by solid boxes, controls are represented by hatched boxes. The horizontal line within the boxes represents the median length, the boxes are the interquartile distance, the whiskers are 1.5 times the interquartile range (IQR) and the circles are observations >1.5 × IQR.

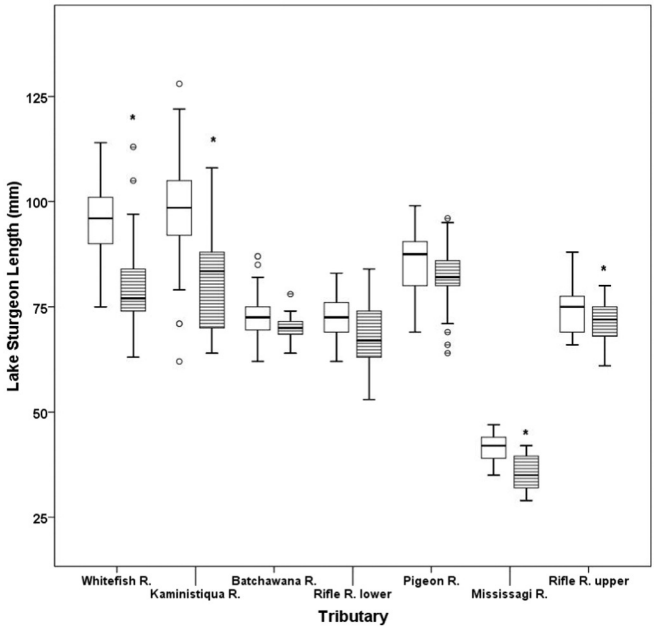

Fig. 4. Box plot comparing lengths (mm) of live and dead lake sturgeon in the treatment section of seven tributaries. The Two-Hearted, Millecoquins, and Sturgeon rivers were not included as individual lake sturgeon lengths were not recorded in these rivers. Live lake sturgeon are represented by solid boxes, dead lake sturgeon are represented by hatched boxes. Dead lake sturgeon were significantly smaller in 4 of the 7 tributaries; marked with *. The horizontal line within the boxes represents the median length, the boxes are the interquartile distance, the whiskers are 1.5 times the interquartile range (IQR) and the circles are observations >1.5 × IQR.

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Table 6
Parameters of the logistic model of lake sturgeon mortality. Alkalinity is mg/L of $CaCO_3$. TFM concentration is measured in mg/L.

	Estimate	S.E.	P-value
Intercept	−17.380	4.911	0.0004
pH	2.838	0.623	5.32E-06
Alkalinity	−0.018	0.003	3.99E-08
Temperature	0.074	0.023	0.0054
TFM Concentration	−0.195	0.089	0.0274
TFM:MLC ratio	−2.091	0.346	1.58E-09

Based on results from previous studies (Johnson et al., 1999; Boogaard et al., 2003), we expected that age-0 lake sturgeon mortality would be >50% in the bioassays and *in situ* exposure to TFM. In our study, lake sturgeon NOECs were at or above sea lamprey MLCs with the exception of three toxicity tests, indicating that lake sturgeon survival *in situ* would, in general, be >50%, and perhaps be as high as 100%, in some instances. These predictions from the bioassay work correspond with our *in situ* observations, where mean lake sturgeon survival in treatment cages was also high at 78.8%, with a wide survival range from 45%–100% observed.

The assumption that hatchery-reared Rainy River strain lake sturgeon respond to lampricide exposure in the same manner as wild Great Lakes lake sturgeon is implicit to this study. The results of this study, as well as results of the Boogaard (2003) study would not be applicable should this be an unfounded assumption. Although we do not have any information regarding differences among strains of lake sturgeon, it is conceivable that hatchery-reared lake sturgeon fed *ad libitum* may be in better condition and have increased survival to lampricide exposure than wild counterparts. Nevertheless, species at risk limitations for Great Lakes strain lake sturgeon means that comparison among strains or direct testing of Great Lakes strain lake sturgeon is not possible, making this a required assumption.

The addition of niclosamide to TFM in high alkaline or high discharge streams reduces the amount of TFM required by increasing the toxicity of TFM to sea lampreys (Gutreuter and Boogaard, 2007). For example, the addition of niclosamide in the lower Rifle River reduced the sea lamprey MLC from 3.6 mg/L for TFM only to 2.1 mg/L for TFM/niclosamide mixture. Consequently, although the addition of niclosamide is not explicitly contained within the survival model, the reduction in the absolute amount of TFM applied to the stream due to the addition of niclosamide results in greater lake sturgeon survival. For example, in

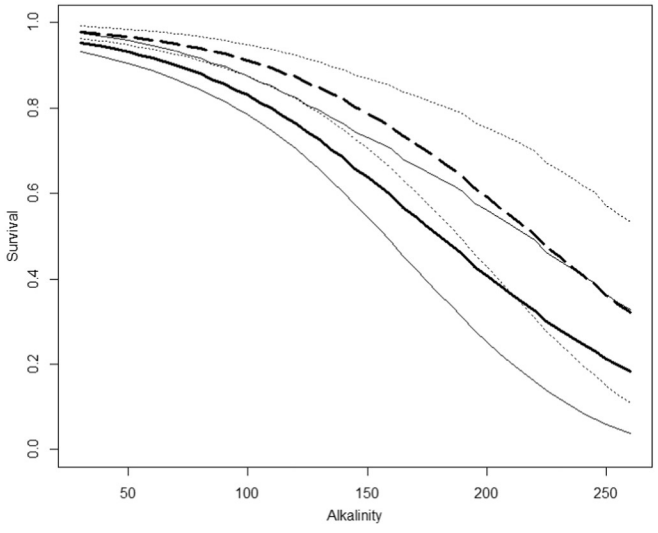

Fig. 5. Lake sturgeon survival profile with 95% confidence intervals, based on alkalinity-specific MLC values at pH 8.1 (mean pH in the study), temperatures of 15 °C (solid lines) and 25 °C (dashed lines), and application at 1.4 × MLC.

Table 7
Forecasts of lake sturgeon survival for pH 8.1 and temperature of 18 °C (mean of *in situ* streams) and increasing alkalinity mg/L $CaCO_3$, with MLC values (mg/L) selected from the pH/alkalinity charts used to set treatment targets. Application concentration of 1.4 × MLC was used in the modeling.

Alkalinity	TFM only			TFM/1% niclosamide		
	Chart MLC	Application Target	Survival	Chart MLC	Application Target	Survival
30	1.4	1.96	0.956	0.9	1.26	0.962
60	1.9	2.66	0.918	1.2	1.68	0.931
90	2.4	3.36	0.850	1.5	2.10	0.879
150	3.5	4.90	0.738	2.2	2.94	0.791
180	3.7	5.18	0.588	2.3	3.08	0.671
210	3.8	5.32	0.441	2.4	3.22	0.536
240	4.1	5.74	0.309	2.6	3.36	0.396
260	4.2	5.88	0.194	2.6	3.50	0.266

treatments at a pH of 8 and an alkalinity of 100 mg/L (the average pH and alkalinity for this study), the TFM MLC is 2.3 mg/L, while the TFM/1% niclosamide MLC is 1.4 mg/L. This is consistent with Boogaard et al. (2003), who found that the addition of 1% niclosamide to a TFM treatment reduced the absolute toxicity of the treatment to lake sturgeon < 100 mm.

Lampricide-induced lake sturgeon mortality was previously found to be size-dependent, with sturgeon smaller than 100 mm having greater mortality than larger cohorts (Boogaard et al., 2003). Our study supports this previous observation to a degree, as mortality of smaller lake sturgeon was higher in four of the seven *in situ* treatments (Fig. 4). Nevertheless, the Mississagi River results are particularly notable, as lake sturgeon survival was high in both the treatment (83%) and bioassay (100%) despite a mean length of only 40 mm in this low alkalinity stream. The lack of a length variable in the predictive model may be a result of a number of factors. The logistic model uses mean length per cage rather than individual lengths as a covariate, and so there may be a lack of contrast within and among streams to further support the length effect observed in other studies. Because application of lampricides in some tributaries is only scheduled after age- 0 lake sturgeon are expected to be >100 mm (Adair and Sullivan, 2009), lake sturgeon length is an important factor in the intersection of sea lamprey and age-0 lake sturgeon management, and the effect of lake sturgeon length should be further evaluated *in situ*.

The current prediction charts for the application of lampricides were developed by Bills et al. (2003) based on bioassays that investigated the effect of pH and alkalinity on toxicity of TFM to sea lampreys and brown trout (*Salmo trutta*), where it was determined that pH was also a critical variable affecting toxicity when applying lampricides to streams. Increasing pH reduces the amount of bioavailable TFM in a stream (Hubert, 2003), and the positive coefficient for the pH term in our model supports this observation. Based on our modeling and empirical results, alkalinity is also an important factor influencing lake sturgeon survival, and survival was lowest in the most alkaline tributaries (Table 7). For example, both the Rifle and Pigeon rivers are high alkalinity tributaries to the Great Lakes, and these tributaries had the lowest *in situ* lake sturgeon survival. Our model demonstrated that as alkalinity increased, predicted survival for lake sturgeon decreased in both TFM and TFM/niclosamide treatments, with greater impacts observed during TFM only treatments (Fig. 5, Table 7). The relationship between alkalinity and lake sturgeon survival during stream treatments should be examined further in both bioassays and *in situ*.

The establishment of MLC and subsequent application rate for a stream treatment is sensitive to measures of pH. For example, assuming an alkalinity of 120, a difference in pH of 0.1 units around pH 8.0 (*e.g.* 7.9, 8.0, and 8.1), translates to an MLC of 2.3, 2.6, and 3.0, respectively. Although pH meters are regularly calibrated to standard ionic buffers during bioassays and lampricide applications (Adair and Sullivan, 2009), the manufacturer specifies an accuracy of ±0.1 pH units on a

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calibrated pH meter, indicating that measurement error in pH could have a significant effect on prescribed MLC. Moreover, the diurnal cycle of pH in stream water can vary significantly during a lampricide application. In this study the pH in Batchawana River varied from 7.58 to 7.82 over an eight hour period, and from 8.13 to 8.69 over a 12-h period in the Millecoquins River. Weise (1984) reported pH values that varied from 8.0 to 8.7 at a lampricide application point, but varied from 7.9 to 9.1 at a sampling site further downstream. Fluctuations in pH can be dramatic; pH changed from 7.03 to 9.05 in Bolton Creek, Ontario, over a 24-h period (DFO unpublished data). Consequently, diurnal pH fluctuation overwhelms potential measurement error of the pH meter, and choice of MLC is based on the expected pH cycle from current data, insight about pH fluctuation from previous lampricide applications, timing of application and instream distribution of lampricides, and the risk aversion of the treatment supervisor.

Lampricide is often applied at a rate greater than chart-predicted MLC to account for attenuation due to ground water, inflowing tributaries, and binding of the active ingredients to sediment as the chemical is transported downstream (Hubert, 2003). As evidence, one-half the treatments in this study were initiated at rates greater than the expected 1.5 × MLC. As mentioned, this increase in application rate is largely a subjective decision, based on the known fate of lampricide blocks during previous treatments, efficacy of treatment, and risk to non-target species. The importance of being judicious when making this decision, as well as the decision whether to include niclosamide, is illustrated by the results from the Pigeon River bioassay and treatment. The Pigeon River is typically treated with both TFM and niclosamide, and the bioassay was set up accordingly. Although the chart-based MLC for TFM/1% niclosamide was 2.6 mg/L, we observed an MLC for sea lamprey larvae of 3.01 mg/L, and NOEC and LC50 values for lake sturgeon of 3.68 and 4.09 mg/L, respectively. In the time between the bioassay and the scheduled treatment, a large rain event caused the river discharge and water chemistry to change, making the river unsuitable for the addition of niclosamide. As a result, the Pigeon River was treated with TFM alone, and the target MLC was 5.2 mg/L, almost twice the amount of the bioassay target, which was based on the expected use of TFM/1% niclosamide. To account for attenuation and dilution, the TFM target concentration at the primary application point was 7.1 mg/L, 1.37 × the chart MLC. Subsequently, lake sturgeon survival was only 24% in the 11 cages in the upper section of the river. Following the expected attenuation and dilution of the lampricide block, TFM concentration dropped to 6.7 mg/L when measured approximately 6 km below the application point, and remained at this concentration for the remainder of the river. Coupled with an increase in stream pH that increased the chart MLC to 6.5 mg/L, lake sturgeon survival increased to 72% for the 9 cages in the lower river. The Pigeon River treatment illustrates the challenges in applying lampricides to streams to effectively control sea lampreys, as well as some of the risks and final decisions required to be made in a short time frame under varying environmental conditions.

Our results indicate that low alkalinity tributaries treated with TFM or TFM/niclosamide have minimal risk to age-0 lake sturgeon, while recruitment in the year of treatment is more likely to be impacted in high alkalinity streams that are treated with TFM. This leads to the question of whether the additional mortality on age-0 lake sturgeon from exposure to lampricides could impair lake sturgeon recovery. Two separate stage-based lake sturgeon recovery modeling studies have identified reducing high natural mortality of age-0 lake sturgeon (Sutton et al., 2004; Vélez-Espino and Koops, 2008) as important for lake sturgeon recovery. They also found that survival of the sub-adult and adult sturgeon life stages had the most influence on long-term population recovery. Alternatively, sea lamprey induced mortality on juvenile and adult lake sturgeon, as estimated from observed wounding rates, resulted in decreases in abundance and reproductive potential of lake sturgeon population (Sutton et al., 2004) and that even a single sea lamprey attack can result in mortality of a lake sturgeon host (Patrick et al., 2009; Sepulveda et al., 2012). This suggests that management actions, such as sea lamprey control, that protect sub-adult and adult life stages from parasitic sea lamprey induced mortality are the most beneficial for lake sturgeon recovery. The net effect of lampricide treatments on lake sturgeon populations at the population level can likely only be understood with additional modeling based on the empirical mortalities observed in this study.

In summary, we found that the majority of age-0 sturgeon (35–120 mm) survived acute exposure to the lampricides TFM and TFM/niclosamide. In the bioassay study, overall lake sturgeon survival at expected sea lamprey MLC was high, with 8 of the 11 bioassays having 100% lake sturgeon survival. *In situ* survival in lampricide treatments ranged from 45 to 100%, with mean survival > 78.8% for lake sturgeon smaller than 100 mm. Our logistic model indicated that this variability in survival depended on factors that affected the absolute TFM concentration exposure of lake sturgeon. There have been recent high-profile age-0 lake sturgeon mortality events (*e.g.*, the Muskegon River treatment noted above) in conjunction with lampricide treatments, and it is our expectation that the logistic model developed here can be used to help predict and potentially moderate these mortality events if the appropriate water chemistry parameters and target lampricide amounts are determined prior to treatment initiation. We believe that this work requires follow-up along to two lines of research. First, laboratory trials have demonstrated that sea lamprey parasitism can result in mortality in adult and sub-adult lake sturgeon (Patrick et al., 2009), so we recommend that stage-based life history models be used to explore the trade-off between age-0 mortality and adult survival in lake sturgeon given the new data presented herein. Second, further research into why high alkalinity streams increase lake sturgeon mortality during a lampricide treatment is required. Following these approaches will help managers achieve the necessary balance between controlling the invasive sea lamprey, a fish that causes millions of dollars of harm in the Great Lakes annually, and restoring lake sturgeon, an iconic Great Lakes species.

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